Electrochemical integration of algal biodiesel production and yeast based fermentation for power production

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Bio-refineries as a concept are focused on the production of chemical energy carriers and, in certain cases, electrical power as well. The production of electrical power, however, is done via thermochemical processing which an inherently inefficient process. Here an electrochemical means of electrical power production is proposed alongside the production of ethanol and biodiesel using yeast and algae respectively. The microbes interact with each other in a microbial fuel cell (MFC) consisting of acid treated Montmorillonite clay (Activated Bleaching Earth) as electrolyte instead of expensive proton exchange membranes. The breakdown of substrate on the anode by yeast provides protons to the algae on the cathode. Experimental evidence proving the efficacy of the concept is provided using an electrochemical cell with salt bridge architecture.

Keywords: Microbial Fuel Cell; Bio-cathode; Fermentation; Activated Bleaching Earth.

Abbreviations: MFC: Microbial fuel cell; PEMFC: Proton exchange membrane fuel cell; MEC: Microbial electrolysis cell; tPMET: trans Plasma Membrane Electron Transport frameworks; PMOR: Plasma Membrane Oxido-Reductase frameworks; CEC: Cation exchange capacity; OCV: Open circuit voltage.

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Introduction

Biodiesel and bio-ethanol are two of the most common fuels produced from biomass technologies. Anaerobic fermentation is a crucial technology which allows economic transformation of cellulosic biomass to valuable fuel and other valuable products [1]. Similarly, biodiesel production from algae is a promising source of renewable higher hydrocarbon based fuels [2]. Algae can yield higher amounts of lipids as compared to vascular plants. Ethanol is also utilized in the transesterification of raw lipids to convert them into fatty acid esters. Utilizing the ethanol from biomass fermentation will allow the process of biodiesel production to become more sustainable. Moreover, the carbon footprint of the process can be reduced by scrubbing the carbon dioxide produced during fermentation by the algae.

To ensure uninterrupted supply of bio-ethanol, the fermentation process should be carried out as close to the photobioreactors as possible. By doing so, the two processes, anaerobic fermentation and algal biodiesel production can be integrated together, thus allowing the sharing of resources and betterment of process economy. Another opportunity is also presented by the proposed integration which can allow us to harness electrical power. The fermentation broth can provide a potential gradient compared

to the algal culture growing under aerobic conditions. This potential gradient can be harnessed in microbial fuel cells.

A microbial fuel cell is a device that converts the chemical energy of organic matter into electricity. Most MFCs use anaerobic bacteria at the anode for the oxidation of the organic matter and transfer the electrons thus produced to the anode from where they are transferred to the cathode and are used to reduce protons. Baker's yeast can also be used as the active media in the anode chamber. While on the cathode side, the reduction of the protons is facilitated by the presence of noble metal catalysts such as platinum. Addition of yeast extract increases the performance of the cell as it acts as a mediator for electron transfer [3]. Inorganic catalysts are extremely expensive and their use can only be justified for extremely rigorous applications. An alternative approach for these inorganic catalysts is biological catalysts such as hydrogenase and nitrogenase. These enzymes are present in many different microbes including algae [4]. The cathode can therefore be submerged in algal medium to provide catalytic activity. Such bio cathodes therefore hold the key to an economical MFC based system.

An important component of the fuel cell is the electrolyte which provides a path for the protons to pass from the anode chamber to the cathode chamber. The most commonly used electrolyte for PEMFC and MFCs is Nafion. Nafion is an excellent conductor of protons and provides a reliable functioning of the fuel cell [5]. But the cost of the membrane is a hindrance to the large scale application of the fuel cell technology. Among other alternatives, soil has also been utilized as an effective electrolyte for the construction of soil based MFCs. The cation capacity of soil is much lower than that of Nafion which makes it unsuitable for replacing it. Here we suggest the use of a semantic clay, Montmorillonite, as a possible replacement of Nafion. The clay is low cost and can achieve higher proton conductivities than normal soil [6]. By treating with acid, the clay can be activated thus resulting in an enhanced cation exchange activity.

MFCs can achieve very low power densities which has resulted in limitation of the usage of the technology. Here we suggest a simple application for the low power density system which can be coupled with anaerobic fermentation to increase its efficiency.

The use of yeast on an industrial scale can be enhanced by accompanying electricity production. Ethanol is a predominant product of yeast fermentation and electricity production will enhance the productivity of the process. Furthermore, many algal strains are used to produce lipids that are converted to biodiesel. Growth of algae on a microbial cathode can result in increased growth due to dependence on the proton availability. Coupling Yeast fermentation with algal harvesting in photo bioreactors will enhance the fruitfulness of both the processes.

When the biomass for fermentation is replaced by the algal biomass remaining after lipid extraction, the process will be more optimized and sustainable. This will make it a microbial solar cell that will produce not only chemical products, but can provide electricity as well [7]. The main components of the cell include a Yeast based anodic compartment and an Algal based cathodic compartment.

Materials and methods

The fuel cell was a simple salt bridge based cell that contained Montmorillonite clay (Ittehad Chemicals Ltd., Pakistan) as electrolyte. The electrolyte was wrapped in filter paper and dipped in the anode and cathode mediums. Cathode was made of Carbon Paper (Paper 120 - TGP-H-120, Toray Industries Inc., Fuel cell store, College Station, Texas, USA) with active surface area of 6 × 9 = 54 cm^2 while the anode was composed of copper plates with an area of 90 cm^2. The electrodes were connected to copper

wire and an external resistance of 3 KΩ was connected between the two electrodes in order to measure power. The cell was run for a month while its performance was monitored.

Two 500 ml beakers were used as containers for anode and cathode mediums. The yeast culture contained 2 g of *S. cerevisiae* along with 3 g of Granulated Yeast Extract to enhance electron transfer. Sugar molasses were used as the substrate with the final solution brix of 3%. This initial culture was fed with fresh molasses in order to maintain the brix. On the cathode side, *Dictyosphaerium iso 8-6* culture was introduced with a cell count of 1.31×10^4 cells/ml. The indigenous algal specie was isolated characterized locally. The algal culture was continuously aerated to avoid the algae from settling down.

The voltage of the system was monitored by using a digital multimeter. The readings were taken in mV. In order to find out the current, a 3k Ohm resistor was connected to the system. Using the value of voltage, the current was found out following Ohm's law.

$$I=V/R$$

The polarization and power curve were plotted by manually varying the external resistance of the cell and calculating the voltage. The value of voltage was then used to find the current and subsequently the power density using the equation:

$$P=V\times I$$

Internal resistance of the cell was calculated using the Ohm's law.

$$\varepsilon=I(R+r)$$

$$V_R=-rI+\varepsilon$$

Results

Effect of time on the open circuit voltage

The cell was observed to mature over a period of a month as the biofilm started to develop on the carbon paper electrode. After 30 days, the cell obtained a maximum OCV and then the value started to decrease. This decrease in the OCV can be because of the deterioration of the biofilm deposited on the electrodes after which a constant OCV was achieved (0.6 V). This change in the OCV is demonstrated in figure 1. This gradual increase in the voltage can be attributed to the development of biofilm on top of the electrode. The copper electrode gained a layer of rust over time which may have contributed in the creation of favorable conditions for the yeast to transfer electrons.

Charge transport

The electrons need to be transported from the medium to the anode to create potential. This transfer of electrons is carried out with the help of addition of mediators. The mediator added here was yeast extract. Still there is a lag between charge creation in the medium and the transfer of the charge to the surface of the anode. This results in a slow response form the cell. A long time is required to achieve the maximum potential, which is roughly 5 minutes. The rise in the voltage is shown in figure 2. About 98% of the maximum voltage (490 mV) is achieved within 2 minutes after which the charge transfer is slowed down. This slowing down of charge transfer is because of increase in the negative potential of the anode which repels electrons. Because of this phenomenon, all the readings of voltage taken required a settling time of 2 minutes.

Performance curves

The power curve of for the fuel cell was plotted and gave a peak of 1.04 mW/m2 at a current density of 5.75 mA/m2. A linear trend was observed in the V-I curves at lower values of resistances. This response is in coherence with the performance of previously demonstrated fuel cells (figure 3).

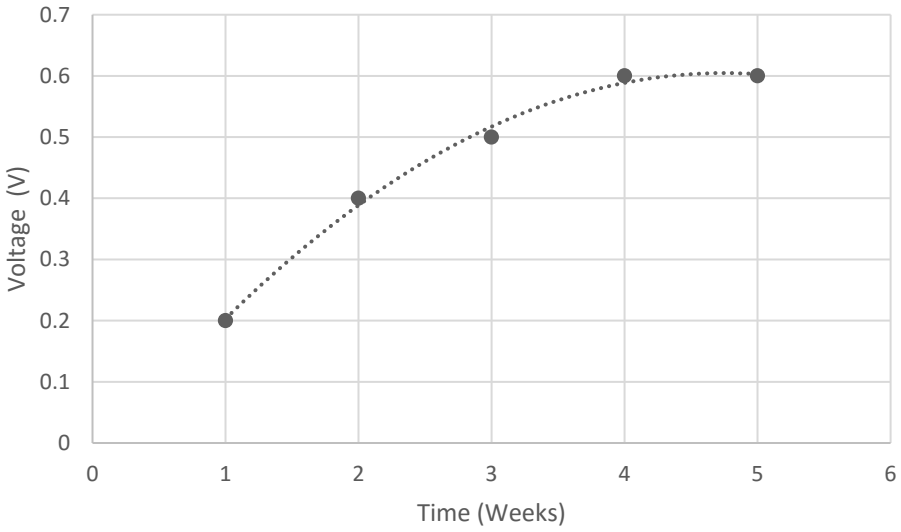

Figure 1. Development of the open circuit voltage of the MFC over a month.

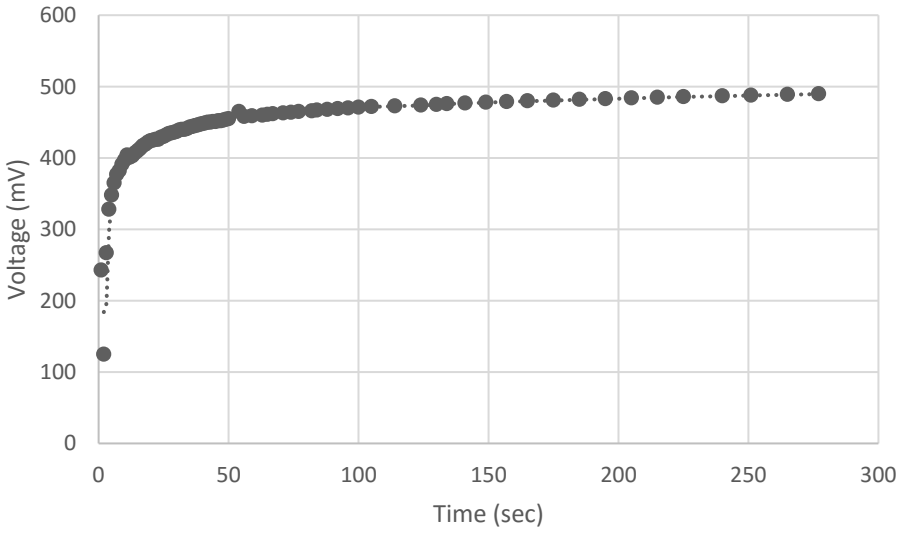

Figure 2. Time required for the MFC to achieve maximum open circuit voltage.

V-R Curve

A second order quadratic equation represents the relation between the external resistance and the output voltage of the cell (figure 4). The relation between voltage and current is not linear and this lag in the increase of voltage can be explained by the mass transport restrictions. Microbes are unable to create the potential with a pace fast enough to keep up with discharge.

Internal resistance

The internal resistance of the system needs to be high for the electronic current to be small. The relation between voltage and current of the cells (figure 5) shows the internal resistance to have a value of 5,500 Ohm. The same equation also gave the value of the emf of the cell, by projecting the graph to the point where current is 0, which was around -464.8 mV. This value of

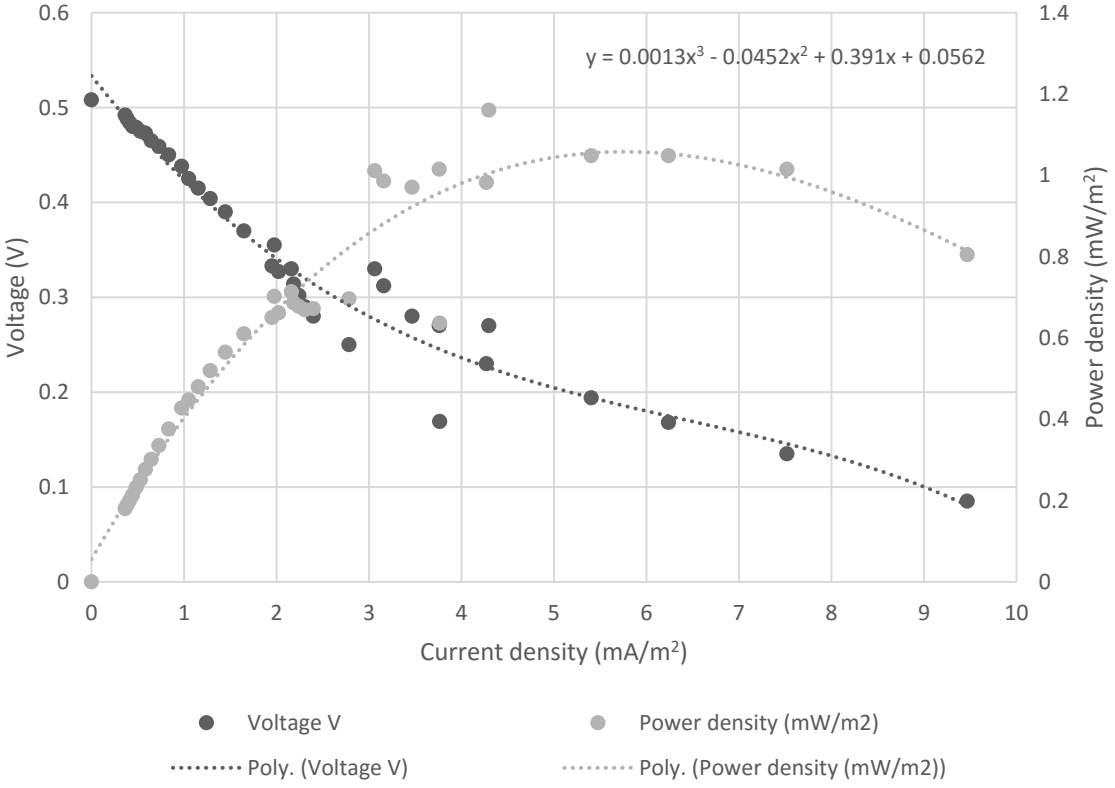

Figure 3. Performance curve of the MFC.

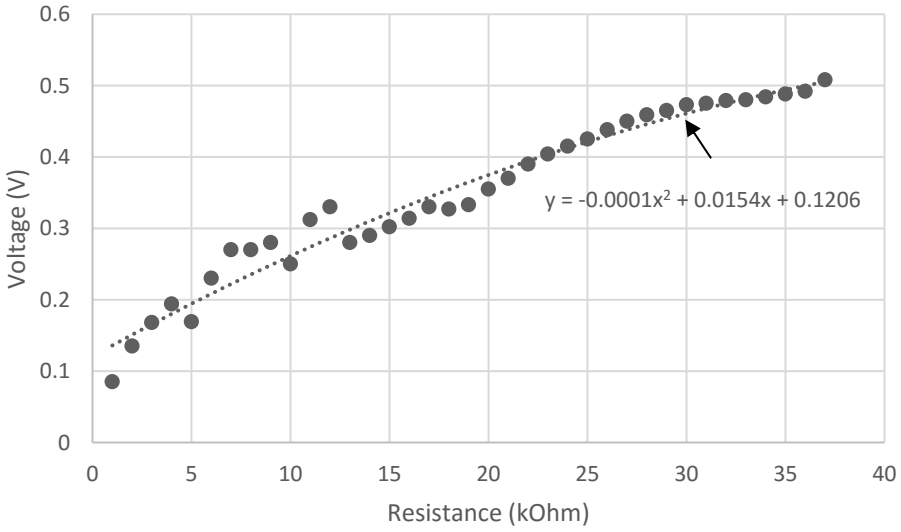

Figure 4. Voltage response of the MFC with change in the external resistance.

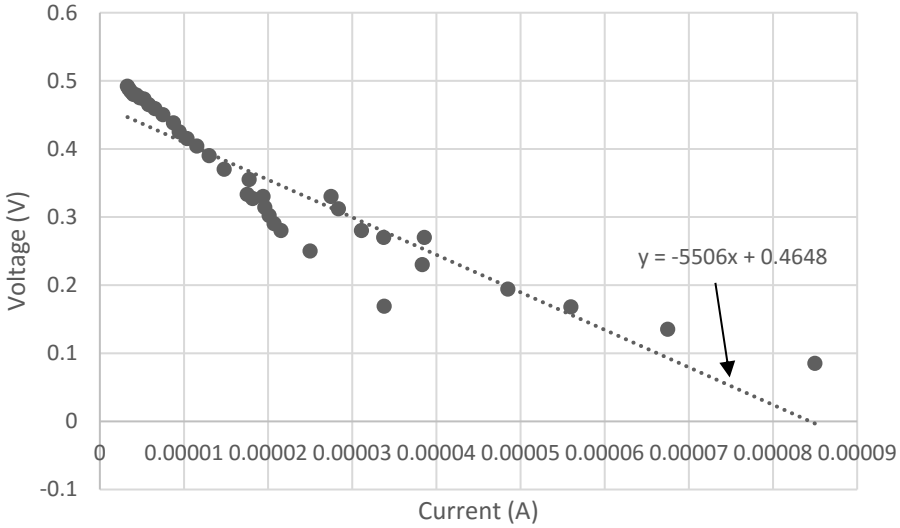

Figure 5. Current-Voltage plot for the MFC used for calculating the internal resistance.

emf can provide us with the cathode potential by using the standard potential for glucose i.e. -428 mV [20].

$$E_{emf} = E_{Cat} - E_{An}$$

$$E_{Cat} = E_{emf} + E_{An} = 464.8 + (-428) = 36.8 \text{ mV}$$

Discussion

Algal bio cathode

Replacement of platinum catalyst on the cathode side is a reason for the usage of bio cathode. Algal cultures have been used as cathodic catalysts after enrichment in the anodic compartment of MFCs. A fuel cell catalyzed by the culture of *Desulfovibrio vulgaris* produced 1.1 A/m^2. Hydrogen production was also demonstrated with a bio cathode. The molasses fed bio electrode was successively fed with bicarbonate and hydrogen and after a few days the hydrogen supply was stopped and the potential of the electrode was lowered which resulted in electron consumption and hydrogen production [8].

In an MEC the sole electron acceptor are protons which reduce to form hydrogen gas. The microbial communities present on the cathode contain hydrogenases that can reversibly reduce protons. Purified hydrogenases have also been used as a catalyst [9].

Bio cathodes make it possible for materials other than oxygen to act as the electron acceptor. They contain bacterial colonies that can catalyze the reduction of multiple compounds. The added benefit of bio cathodes is the possibility of achieving the production of value added products through electrochemical reduction. The electron transfer from the electrode to the microbe can take place either directly or indirectly. The indirect transfer of electrons depends on the use of some mediator. The electron acceptors in the cells are mainly complexes like cytochromes. The development of a biofilm on the electrode with the use of oxygen as the electron acceptor is beneficial. The increase in the thickness of the biofilm was observed to reduce the electrical production. Increase in the electrode area is also shown to increase the catalytic activity of the microbes and hence increase the utilization of the electrons coming from the anode [10].

The anode of acetate oxidizing tubular microbial fuel cell was combined with an open-air bio

cathode for electricity production. Increasing the size of the cell reduced the volumetric power production [11].

The function of algae on the bio cathode in an MFC is to reduce the oxygen present and combine it with the protons flowing from the anode side. In this way, the anode side reactions can be summarized as

Substrate → Carbon dioxide + Protons + Electrons

Meanwhile on the cathode side

$$\text{Protons} + \text{Oxygen} + \text{Electrons} \xrightarrow{\text{Algae}} \text{Water}$$

In such a system, algae are used as a source of oxygen for the cathode. The level of dissolved oxygen therefore matter a lot and will vary throughout the day. The power output of such a system is very small and only a few micro watts are produced per meter sq. [12].

Yeast microbial fuel cell

Saccharomyces cerevisiae has been used as the biocatalyst to oxidize glucose. The catalytic activity came from the cells adhered to the anode and the dispersed cells did not seem to take part in electron transfer. There are many advantages to using Baker's yeast including its low cost, availability, easy dry storage and mass cultivation [13]. On the other hand, this easy to grow yeast has also been used to ferment molasses. The main product of this fermentation process is ethanol which can be purified to be used as fuel [14]. The experiment that we have devised shows that this fermentation process can be coupled with algal bio cathode to produce additional power.

Yeast is thought to be perfect biocatalysts for microbial energy units which can utilize a wide range of substrates. Along with this, they are mostly nonpathogenic which can help in creating less hazardous cells. Yeasts are said to have two mechanisms for electron transfer i.e. trans Plasma Membrane Electron Transport frameworks (tPMET) & Plasma Membrane Oxido-Reductase frameworks (PMOR). Collectively these mechanisms result in enhanced electron transfer [15].

Copper is considered to be a toxic metal for bacteria. Even small traces of the metal can harm them [16]. But the toxicity of copper is irrelevant in bio electrochemical devices and copper can produce much higher current densities owing to its high conductivity [17].

Electrolyte

The electrolyte used was Activated Bleaching Earth which is a semantic mineral namely Montmorillonite. This mineral was used as a proton exchange medium because of its inherent cation exchange capacity (CEC) [18]. Montmorillonite is classified as a Phyllosilicate because it contains two tetrahedral sheets of silica which sandwich an octahedral sheet of alumina. Along with alumina, MgO is also found in the middle layer which can impart cation exchange capacity to the clay by isomorphous substitution. The composition of the clay is shown in table 1. When these ions move a net negative charge remains on the clay because of oxygen atoms. This allows positive charge to flow through the clay [19].

Table 1. Chemical composition of Montmorillonite clay.

SiO2 %	55 ~ 80
Al2O3 %	5 ~ 20
Fe2O3 %	2 ~ 10
MgO %	0 ~ 8
CaO %	0 ~ 5
Na2O %	0 ~ 2
K2O %	0 ~ 2

Application

The yeast is used to oxidize molasses and produce ethanol. Our experiment has shown that electricity can also be produced during the fermentation process. Algae is grown in photo bioreactors and harvested to produce biodiesel. This algae can also be used in the bio cathode of a microbial fuel cell.

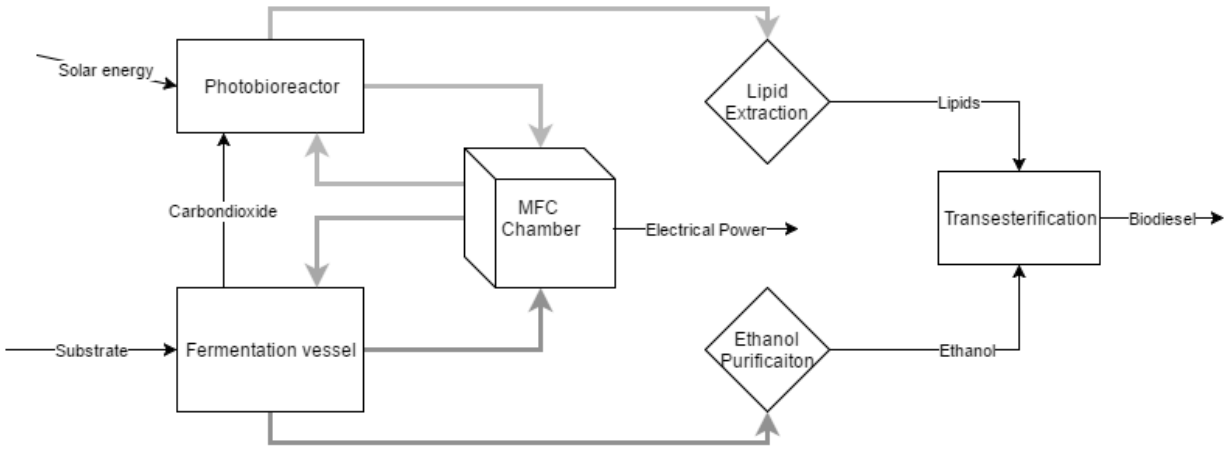

Figure 6. Flow diagram of the proposed combined algal growth and fermentation system.

A configuration that allows these two microbes to work together is described here. It involves the flow of the liquids in microporous tubes. These tubes are embedded in ABE which acts as the ion exchange medium and transfers protons from the yeast to the algae. Copper wires are to be passed in the middle of the tubes carrying yeast while carbon paper electrodes will be present on the algae side. The large amount of clay present in the container will ensure the separation among the fluids.

A constant flow rate is to be maintained for the algal culture and the fermentation broth as it passes through the MFC chamber. Simultaneously the algal culture can be harvested for lipid extraction and the fermentation broth is subjected to ethanol purification. The ethanol is used for transesterification and production of biodiesel. figure 6 shows the flow diagram of the algal and yeast cultures starting from the major input of a substrate to eventual production of biodiesel as a primary product.

The cation exchange capacity of Montmorillonite is between 70 – 100 meq/100 g [6]. Nafion has an equivalent weight of 1,100, which translates into an ion exchange capacity of 900 meq/100 g. But up to 25% of the sites are unassailable which reduces the capacity of Nafion which is still significantly higher than that of ABE [21]. This difference in capacity is overcome by the difference in cost of the two materials.

The results show that with some additional refinement this fuel cell can be used on a large scale to produce power along with industrial fermentation. The anode area needs to be sufficient in order to collect as many electrons as possible. This will ensure an increase in the energy recovery from the fermentation process whereby substrate utilization will be maximized. For the algal bio cathode, this system can provide an additional usage of the algal biomass before being converted to biodiesel.

The most energy intensive step involved in the biodiesel production process is of algae separation from the culture. This process is mostly carried out by flocculation brought upon by increase in the pH of the culture which, along with sedimentation are the two of least energy intensive processes, whit the appended disadvantage of low efficiencies and higher time consumption. On the other hand, centrifugation and ultrafiltration are efficient technologies which require massive amounts of energy input. Increase in the efficiencies of passive processes and decreasing the time requirement can allow the process to become economically feasible for implementation on a grander scale. This

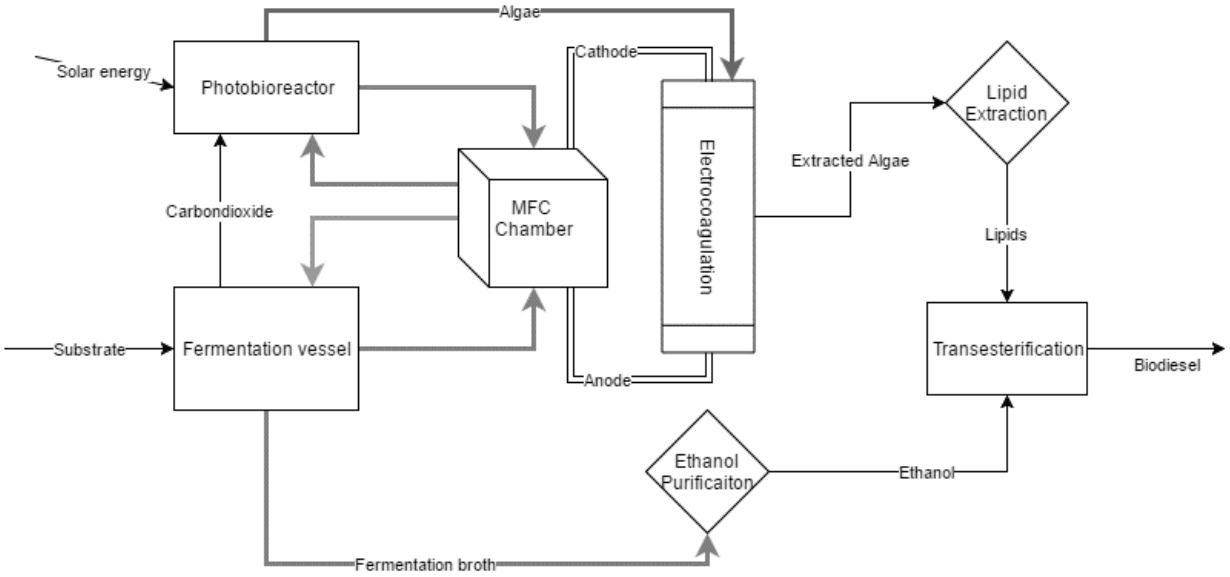

Figure 7. Flow diagram of the proposed combined algal growth and fermentation system along with electrocoagulation.

problem has been addressed by the introduction of electrocoagulation and electroflotation which relies on metallic electrodes to stimulate and enhance the formation of flocs and subsequently better separation [22, 23].

The proposed electrochemical is primarily tested as a MFC for power production but the available potential gradient can be put to another use to enhance the overall process of the biorefinery. Optimization of the voltage for bringing about electrocoagulation or electroflotation of the culture media can cause the economics of the process to shift and warrant its physical implementation. The concept is pictorially represented in figure 7.

Conclusion

The possibility of using fermentation broth along with algal culture in an MFC has been experimentally tested here in a simple electrochemical cell configuration. To avoid the use of expensive components, mainly the proton exchange membrane, activated bleaching earth has been introduced as an electrolyte. The experiment shows that the potential gradient between the algal and yeast cultures can be harnessed even with a low quality and low cost electrolyte. The proposed MFC chamber, with further modifications, can become a valuable addition to the integrated approach towards the production of algal based biodiesel.

Further research looking in to the feasibility and effectiveness of integrating electrochemical technologies into bioprocessing plants is needed to warrant widespread use of renewable biological resources.

Reference

1. Gunaseelan VN. 1997. Anaerobic digestion of biomass for methane production: A review. Biomass and Bioenergy. 13: 83–114.
2. Mata TM, Martins AA, and Caetano NS. 2010. Microalgae for biodiesel production and other applications: A review. Renew. Sustain. Energy Rev. 14: 217–232.
3. Sayed ET, Barakat NAM, Abdelkareem MA, Fouad H, and Nakagawa N. 2015. Yeast Extract as an Effective and Safe Mediator for the Baker's-Yeast-Based Microbial Fuel Cell. Ind. Eng. Chem. Res. 54: 3116–3122.
4. Lubitz W, Ogata H, Ru O, and Reijerse E. 2014. Hydrogenases. Chemical Reviews 114: 4081-4148.

5. Chae KJ, Choi M, Ajayi FF, Park W, Chang IS, and Kim IS. 2008. Mass Transport through a Proton Exchange Membrane (Nafion) in Microbial Fuel Cells. Energy & Fuels. 22: 169–176.
6. Carrol D. 1959. Ion Exchange in Clays and other Minerals. Geol. Soc. Am. Bull. 70: 749.
7. Strik DPBTB, Timmers RA, Helder M, Steinbusch KJJ, Hamelers HVM, and Buisman CJM. 2011. Microbial solar cells: applying photosynthetic and electrochemically active organisms.Trends Biotechnol. 29: 41–9.
8. Croese E, Pereira MA, Euverink GJW, Stams AJM, and Geelhoed JS. 2011. Analysis of the microbial community of the biocathode of a hydrogen-producing microbial electrolysis cell. Appl. Microbiol. Biotechnol. 92: 1083–93.
9. Lojou E. 2011. Hydrogenases as catalysts for fuel cells: Strategies for efficient immobilization at electrode interfaces. Electrochim. Acta. 56: 10385–10397.
10. Huang L, Regan JM, and Quan X. 2011. Electron transfer mechanisms, new applications, and performance of biocathode microbial fuel cells. Bioresour. Technol. 102: 316–323.
11. Clauwaert P, van der Ha D, Boon N, Verbeken K, Verhaege M, Rabaey K, and Verstraete W. 2007. Open Air Biocathode Enables Effective Electricity Generation with Microbial Fuel Cells. Environ. Sci. Technol. 41: 7564–7569.
12. González del Campo A, Cañizares P, Rodrigo MA, Fernández FJ, and Lobato J. 2013. Microbial fuel cell with an algae-assisted cathode: A preliminary assessment. J. Power Sources. 242: 638–645.
13. Sayed ET, Tsujiguchi T, and Nakagawa N. 2012. Catalytic activity of baker's yeast in a mediatorless microbial fuel cell. Bioelectrochemistry. 86: 97–101.
14. Lin Y and Tanaka S. 2006. Ethanol fermentation from biomass resources: current state and prospects. Appl. Microbiol. Biotechnol. 69: 627–42.
15. Schaetzle O, Barrière F, and Baronian K. 2008. Bacteria and yeasts as catalysts in microbial fuel cells: electron transfer from micro-organisms to electrodes for green electricity. Energy Environ. Sci. 1: 607.
16. Logan BE, Hamelers B, Rozendal R, Schröder U, Keller J, Freguia S, Aelterman P, Verstraete W, and Rabaey K. 2006. Microbial Fuel Cells: Methodology and Technology. Environ. Sci. Technol. 40: 5181–5192.
17. Baudler A, Schmidt I, Langner M, Greiner A, and Schröder U. 2015. Does it have to be carbon? Metal anodes in microbial fuel cells and related bioelectrochemical systems. Energy Environ. Sci. 8: 2048–2055.
18. Pan C and Shen YH. 2003. Estimation of Cation Exchange Capacity of Montmorillonite by Cationic Surfactant Adsorption. Commun. Soil Sci. Plant Anal. 34: 497–504.
19. Gieseking JE. 1939. The Mechanism of Cation Exchange in Montmorillonite-Beidellite-Nontronite type of clay minerals. Soil Sci. 47: 1–14.
20. Logan BE. 2008. Microbial fuel cells. John Wiley & Sons.
21. Chen TY and Leddy J. 2000. Ion Exchange Capacity of Nafion and Nafion Composites. Langmuir. 16: 2866–2871.
22. Baierle F, John DK, Souza MP, Bjerk TR, Moraes MSA, Hoeltz M, Rohlfes ALB, Camargo ME, Corbellini VA, and Schneider RCS. 2015. Biomass from microalgae separation by electroflotation with iron and aluminum spiral electrodes. Chem. Eng. J. 267: 274–281.
23. Tumsri K, and Chavalparit O. 2011. Optimizing electrocoagulation-electroflotation process for algae removal. In 2nd International Conference on Environmenta l Science and Technology IPCBEE (Vol. 6).